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(54) Title: ANTAGONISTS TO GROWTH ARREST SPECIFIC GENE 6 TO TREAT INSULIN-RESISTANT DISORDERS

(57) Abstract

An antagonist to the activator of the Rse and Mer receptor protein tyrosine kinases, encoded by growth arrest–specific gene 6 (gas6), is found to be useful in a method of treating an insulin–resistant disorder such as diabetes. More particularly, a method for treating an insulin–resistant disorder is provided which comprises administering to a mammal in need of such treatment an effective amount of a composition comprising a gas6 antagonist. A hypoglycemic agent may be co–administered with the gas6 antagonist.

Insulin-resistant disorders

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ANTAGONISTS TO GROWTH ARREST SPECIFIC GENE 6 TO TREAT INSULIN-RESISTANT DISORDERS

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates generally to a method of treating insulin-resistant disorders such as type II diabetes. More particularly, the invention relates to methods of treating insulin-resistant patients using an antagonist to gas6, which is a ligand to the Rse receptor, the Mer receptor, and the Axl receptor.

Description of Related Art

Specific signals that control the growth and differentiation of cells in developing and adult tissues often exert their effects by binding to and activating cell surface receptors containing an intrinsic tyrosine kinase activity. The human and murine complementary DNA sequences of the receptor tyrosine kinase Rse are preferentially expressed in the adult brain. Mark *et al.*, J. Biol. Chem., 269: 10720 (1994). The extracellular domain of Rse receptor is composed of two immunoglobulin-like (Ig-L) repeats followed by two fibronectin type III repeats. Complementary DNA sequences encoding proteins identical to human (Ohashi *et al.*, Oncogene, 9: 699 (1994)) and murine Rse (Lai *et al.*, Oncogene, 9: 2567 (1994)) have been reported independently, and termed Sky and Tyro3, respectively. See also Fujimoto and Yamamoto, Oncogene, 9: 693 (1994) concerning the murine equivalent to Rse, designated brt, and Dai *et al.* Oncogene, 9: 975 (1994) concerning the human equivalent, designated tif.

The expression of Rse in various tissues has been investigated. Lai *et al.*, *supra*, found that, in the adult brain, Rse mRNA is localized in neurons of the neocortex, cerebellum and hippocampus. Other investigators similarly found that Rse is expressed at high levels in the cerebral cortex, the lateral septum, the hippocampus, the olfactory bulb and the cerebellum. The highest levels of Rse expression in the brain were found to be associated with neurons. Schulz *et al.* Molec. Brain Res., 28: 273-280 (1995). In the central nervous system (CNS) of mice, the expression of Rse is detected at highest levels during late embryonic stages and post birth, coincident with the establishment and maintenance of synaptic circuitry in cortical and hippocampal neurons. Lai *et al.*, *supra*, and Schneider *et al.*, Cell, 54: 787-793 (1988). This process is believed to be regulated locally, by cells that are in direct contact or positioned close to one another. By Northern blot analysis, Mark *et al.*, *supra*, found that high levels of Rse mRNA were present in samples of RNA from the brain and kidney.

Dai *et al.*, *supra*, found that Rse was highly expressed in human ovary and testes. The expression of Rse in various human cell lines was also analyzed by Mark *et al.*, *supra*. Little, or no, Rse mRNA was detected by Northern blotting of mRNA samples from the monocyte cell line THP-1 or the lymphoblast-like RAJI cells. However, the Rse transcript was detected in a number of hematopoietic cell lines, including cells of the myeloid (*i.e.*, myelogenous leukemia line K562 and myelomonocytic U937 cells) and the megakaryocytic leukemia lines DAMI and CMK11-5, as well as the human breast carcinoma cell line MCF-7. In the cell lines examined, the highest level of expression was observed in Hep 3B cells, a human hepatocarcinoma cell line.

Rse is structurally related to Axl (also known as Ufo or Ark) and shares 43% overall amino acid sequence identity with this tyrosine kinase receptor. See O'Bryan *et al.*, Mol. Cell. Biol., 11: 5016 (1991), Janssen *et al.*, Oncogene, 6: 2113 (1991), Rescigno *et al.* Oncogene, 5: 1908 (1991) and Bellosta *et al.*, Mol. and Cell. Biol., 15: 614 (1995) concerning Axl. Rse and Axl, together with Mer (Graham *et al.*, Cell Growth Differ., 5: 647 (1994)), define a class of receptor tyrosine kinases whose extracellular domains resemble neural cell recognition and adhesion molecules (reviewed by Ruitishauser, U. in Current Opin. Neurobiology, 3: 709 (1993) and Brummendorf and

Rathjen in J. Neurochemistry, 61: 1207 (1993)). Like Rse, Axl is also expressed in the nervous system, but is more widely expressed than Rse in peripheral tissues.

Mer mRNA is detected in peripheral blood mononuclear cells, in bone marrow mononuclear cells, and in monocytes, but not in granulocytes. Despite the fact that Mer mRNA is expressed in neoplastic B and T cell lines, it is not detected in normal B or T lymphocytes. Mer is widely expressed in human tissues, but the highest levels of Mer mRNA are detected in the testis, ovary, prostrate, lung, and kidney. Graham *et al.*, *supra*.

Disregulated expression of Mer, Rse, and Axl is associated with cellular transformation. For example, Axl was isolated from DNA of patients with chronic myelogenous leukemia (O'Bryan *et al.*, *supra*) and chronic myeloproliferative disorder (Janssen *et al.*, *supra*) using a transfection/tumorogenicity assay. Mer was cloned from a neoplastic B cell line and is expressed in numerous transformed T acute lymphocytic leukemia cell lines (Graham *et al.*, *supra*). Rse and Axl, when overexpressed in fibroblasts, induce cellular transformation. O'Bryan *et al.*, *supra*; Ohashi *et al.*, Oncogene, 9: 669 (1994); Taylor *et al.*, J. Biol. Chem., 270: 6872-6880 (1995); and McCloskey *et al.*, Cell Growth and Diff., 5: 1105-1117 (1994). Rse mRNA and protein are also overexpressed in mammary tumors derived from transgenic animals that overexpress either the *wnt-1* or *fgf-3* oncogenes. Taylor *et al.*, J. Biol. Chem., 270: 6872-6880 (1994).

Putative ligands for the Rse and Axl receptors have been reported. Varnum *et al.* Nature, 373: 623 (1995) and Stitt *et al.* Cell, 80: 661-670 (1995) recently reported that gas6 (for growth arrest-specific gene 6) is a ligand for Axl. Gas6 belongs to a set of murine genes which are highly expressed during serum starvation in NIH 3T3 cells. Schneider *et al.*, Cell, 54: 787-793 (1988). These genes were designated growth arrest-specific genes, since their expression is negatively regulated during growth induction. The human homolog of murine gas6 was also cloned and sequenced by Manfioletti *et al.* in Molec. Cell Biol., 13: 4976-4985 (1993). They concluded that gas6 is a vitamin K-dependent protein and speculated that it may play a role in the regulation of a protease cascade relevant to growth regulation. Gas6 is expressed in a variety of tissues including the brain. See also Colombo *et al.* Genome, 2: 130-134 (1992), Ferrero *et al.* J. Cellular Physiol., 158: 263-269 (1994), Goruppi *et al.*, Oncogene, 12: 471-480 (1996), Mark *et al.*, J. Biol. Chem., 271: 9785-9789 (1996); Li *et al.*, J. Neuroscience, 16: 2012-2019 (1996); U.S. Pat. No. 5,538,861; and WO 96/28548 concerning gas6.

Stitt *et al.*, *supra*, further reported that protein S is the ligand for Tyro3. Protein S is a vitamin K-dependent plasma protein that functions as an anticoagulant by acting as a cofactor to stimulate the proteolytic inactivation of factors Va and VIIIa by activated protein C. See the review by Easmon *et al.* Arterioscler. Thromb., 12: 135 (1992). Accordingly, protein S is an important negative regulator of the blood-clotting cascade. See Walker *et al.*, J. Biol. Chem., 255: 5521-5524 (1980), Walker *et al.*, J. Biol. Chem., 256: 11128-11131 (1981), Walker *et al.*, Arch. Biochem. Biophys., 252: 322-328 (1991), Griffin *et al.*, Blood, 79: 3203 (1992), and Easmon, Arterioscler. Thromb., 12: 135 (1992). The discovery that about half of the protein S in human plasma is bound to C4BP further supports the notion that protein S is involved in the complement cascade. Dahlback *et al.*, PNAS(USA), 78: 2512-2516 (1981). The role of protein S as a mitogen for smooth muscle cells has also been reported. Gasic *et al.*, PNAS (USA), 89: 2317-2320 (1992).

Protein S can be divided into four domains (see Figs. 1A, 1C and 1D of WO 96/28548). Residues 1-52 (Region A) are rich in γ-carboxyglutamic acid (Gla) residues which mediate the Ca^{2+} dependent binding of protein S to negatively charged phospholipids. Walker, J. Biol. Chem., 259: 10335 (1984). Region B includes a thrombin-

sensitive loop. Region C contains four epidermal growth factor (EGF)-like repeats. Region D is homologous to the steroid hormone binding globulin (SHBG) protein. Hammond *et al.*, FEBS Lett. 215: 100 (1987). As discussed by Joseph and Baker (FASEB J., 6: 2477 (1994)), this region is homologous to domains in the A chain of laminin (23% identity) and merosin (22% identity) and to a domain in the *Drosophila* crumbs (19%).

Murine and human gas6 cDNAs encode proteins having 43 and 44% amino acid sequence identity, respectively, to human protein S.

Insulin regulates blood glucose by decreasing glucose outflow from the liver and increasing glucose uptake in peripheral tissues, for example, muscles and adipose tissues. Insulin exerts these effects by interacting with the insulin receptor present on most cells. The sensitivity of a mammal to insulin is a function of the number of insulin receptors of individual cells. This number is down-regulated by insulin, *i.e.*, high concentrations of insulin secondarily lead to relative insulin resistance.

Pathologies in which an excessive endogenous insulin is secreted include obesity, type 2 diabetes, hyperlipidemia, and type IV of Fredricksen. In type 1 diabetes (insulin-dependent diabetes mellitus), insulin resistance is the consequence of the peripheral administration of insulin, so that the glucose hemostatic function of the liver is impaired and peripheral glucose uptake excessive. The treatment of obesity, type 2 diabetes (non-insulin dependent diabetes mellitus), and hyperlipidemia consists primarily of administering insulin and changing dietary behavior. In insulin-treated type 1 diabetes, hyperinsulinemia results from the fact that insulin is delivered subcutaneously rather than intraportally so that the delivered insulin reaches peripheral tissues first rather than after passage through the liver.

Insulin-like growth factor-I (IGF-I) has hypoglycemic effects in humans similar to those of insulin when administered by intravenous bolus injection. Underwood *et al.*, Hormone Research, 24: 166 (1986). IGF-I is known to exert glucose-lowering effects in both normal (Guler *et al.*, N. Engl. J. Med., 317: 137-140 (1987); U.S. Pat. No. 4,988,675) and diabetic individuals (Schoenle *et al.*, Diabetologia, 34: 675-679 (1991); Zenobi *et al.*, J. Clin. Invest., 90: 2234-2241 (1992); Sherwin *et al.*, Hormone Research, 41 (Suppl. 2): 97-101 (1994); Takano *et al.*, Endocrinol. Japan, 37: 309-317 (1990); Guler *et al.*, Acta Paediatr. Scand. (Suppl.), 367: 52-54 (1990)), with a time course described as resembling regular insulin. See also Kerr *et al.*, "Effect of Insulin-like Growth Factor I on the responses to and recognition of hypoglycemia," American Diabetes Association (ADA), 52nd Annual Meeting, San Antonio, Texas, June 20-23, 1992, which reported an increased hypoglycemia awareness following recombinant human IGF-I (rhIGF-I) administration. In addition, single administration of rhIGF-I reduces overnight GH levels and insulin requirements in adolescents with IDDM. Cheetham *et al.*, Clin. Endocrinol., 40: 515-555 (1994); Cheetham *et al.*, Diabetologia, 36: 678-681 (1993).

The administration of rhIGF-I to type II diabetics, as reported by Schalch *et al.*, J. Clin. Metab., 77: 1563-1568 (1993), demonstrated a fall in both serum insulin as well as a paralleled decrease in C peptide levels. This indicated a reduction in pancreatic insulin secretion after five days of IGF-I treatment. This effect has been independently confirmed by Froesch *et al.*, Horm. Res., 42: 66-71 (1994). *In vivo* studies in normal rats also illustrate that IGF-I infusion inhibits pancreatic insulin release. Fursinn *et al.*, Endocrinology, 135: 2144-2149 (1994). In addition, in pancreas perfusion preparations, IGF-I also suppressed insulin secretion. Leahy *et al.*, Endocrinology, 126: 1593-1598 (1990). Despite these clear *in vivo* inhibitory effects of IGF-I on insulin secretion in humans and animals, *in vitro* studies have not yielded such uniform results.

RhIGF-I has the ability to improve insulin sensitivity. For example, rhIGF-I (70 μg/kg bid) improved insulin sensitivity in non-diabetic, insulin-resistant patients with myotonic dystrophy. Vlachopapadopoulou *et al.*, J. Clin. Endo. Metab., 12: 3715-3723 (1995). Saad *et al.*, Diabetologia, 37: Abstract 40 (1994) reported dose-dependent improvements in insulin sensitivity in adults with obesity and impaired glucose tolerance following 15 days of rhIGF-I treatment (25 μg and 100 μg/kg bid). RhIGF-I also improved insulin sensitivity and glycemic control in some patients with severe type A insulin resistance (Schoenle *et al.*, Diabetologia, 34: 675-679 (1991); Morrow *et al.*, Diabetes, 42 (Suppl.): 269 (1993) (abstract); Kuzuya *et al.*, Diabetes, 42: 696-705 (1993)) and in other patients with non-insulin dependent diabetes mellitus. Schalch *et al.*, "Short-term metabolic effects of recombinant human insulin-like growth factor 1 (rhIGF-I) in type II diabetes mellitus", in: Spencer EM, ed., Modern Concepts of Insulin-like Growth Factors (New York: Elsevier: 1991) pp. 705-715; Zenobi *et al.*, J. Clin. Invest., 90: 2234-2241 (1993).

A general scheme for the etiology of some clinical phenotypes that give rise to insulin resistance and the possible effects of administration of IGF-I on selected representative subjects is given in several references. See, *e.g.*, Elahi *et al.*, "Hemodynamic and metabolic responses to human insulin-like growth factor-1 (IGF-I) in men," in: Modern Concepts of Insulin-Like Growth Factors, (Spencer. EM, ed.), Elsevier, New York, pp. 219-224 (1991); Quinn *et al.*, New Engl. J. Med., 323: 1425-1426 (1990); Schalch *et al.*, "Short-term metabolic effects of recombinant human insulin-like growth factor 1 (rhIGF-I) in type 11 diabetes mellitus," in: Modern Concepts of Insulin-Like Growth Factors, (Spencer, EM, ed.), Elsevier, New York, pp. 705-714 (1991); Schoenle *et al.*, Diabetologia, 34: 675-679 (1991); Usala *et al.*, N. Eng. J. Med., 327: 853-857 (1992); Lieberman *et al.*, J. Clin. Endo. Metab., 75: 30-36 (1992); Zenobi *et al.*, J. Clin. Invest., 90: 2234-2241 (1992); Zenobi *et al.*, J. Clin. Invest., 89: 1908-1913 (1992); and Kerr *et al.*, J. Clin. Invest., 91: 141-147 (1993). Methods of chronic amelioration and reversal of insulin resistance obtained by exposing a cell to a modification-effective amount of IGF-I for at least about seven days are disclosed in WO 94/16722. However, when IGF-I was used to treat type II diabetic patients in the clinic at a dose of 120-160 μg/kg twice daily, the side effects outweighed the benefit of the treatment. Jabri *et al.*, Diabetes, 43: 369-374 (1994). See also Wilton, Acta Paediatr., 383: 137-141 (1992) regarding side effects observed upon treatment of patients with IGF-I.

Since some patients are resistant to insulin, there is a need for better therapeutic measures for controlling insulin-resistantstates such as diabetes, particularly type II diabetes. There is a further need for a diagnostic agent that indicates whether a patient is insulin resistant, and hence eligible for such therapy.

SUMMARY OF THE INVENTION

Accordingly, in one aspect, the invention provides a method for treatment of insulin-resistant disorders comprising administering to a mammal in need of such treatment an effective amount of a composition comprising a gas6 antagonist. In a preferred aspect, the disorder is diabetes, more preferably type II diabetes, the mammal is a human, the gas6 antagonist is to human gas6 polypeptide, more preferably native-sequence gas6 polypeptide, and the gas6 antagonist is an antibody to a gas6 receptor, more preferably a human or humanized antibody to a gas6 receptor. In another preferred aspect, an effective amount of a hypoglycemic agent is administered to the mammal, either being present in the composition containing the gas6 antagonist or being administered separately from the gas6 antagonist. More preferably, the hypoglycemic agent is insulin, an IGF, a sulfonylurea, or a thiazolidinedione, still more preferably, insulin or IGF-I, and most preferably insulin.

In another aspect, the invention provides a composition comprising a gas6 antagonist and a hypoglycemic agent, preferably with a carrier such as a physiologically acceptable carrier. In another preferred aspect, the hypoglycemic agent is a thiazolidinedione or sulfonylurea.

In a third aspect, the invention provides an article of manufacture, comprising:

a container;

a label on said container; and

a composition contained within said container comprising a gas6 antagonist; wherein the composition is effective for treating a mammal with an insulin-resistant disorder and the label on said container indicates that the composition can be used for treating an insulin-resistant disorder.

Preferably, in this article the gas6 antagonist is to human gas6 polypeptide and the gas6 antagonist is an antibody against a gas6 receptor, more preferably a human or humanized antibody against a gas6 receptor. Also, in a preferred article of this type, the composition further comprises insulin, and the disorder being treated is diabetes, more preferably type II diabetes.

In a fourth aspect, the invention provides an article of manufacture, comprising:

a first container;

a label on said first container;

a first composition contained within said first container comprising a gas6 antagonist;

a second container;

a label on said second container;

a second composition contained within said second container comprising a hypoglycemic agent; wherein the compositions are effective for treating a mammal with an insulin-resistant disorder and the labels on said containers indicate that the compositions can be used for treating an insulin-resistant disorder.

In a still further embodiment, the invention provides a method for determining if a mammal has an insulin-resistant disorder comprising measuring the level of endogenous gas6 in a body sample of the mammal and ascertaining if the level is elevated over the level in a comparable mammal that does not have an insulin-resistant disorder. In a preferred embodiment, the step of measuring the level of endogenous gas6 is accomplished using an antibody to gas6 in an ELISA or RIA format or method.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

1. Definitions

As used herein, the terms "gas6" and "gas6 polypeptide" (unless indicated otherwise) refer to a polypeptide which is able to activate the Rse receptor, Mer receptor, or Axl receptor and encompass the mature, pre-, prepro- and pro- forms of gas6 polypeptide, either purified from a natural source, chemically synthesized or recombinantly produced. The present definition specifically includes "human" gas6 polypeptide comprising the amino acid sequence published in Manfioletti *et al.*, *supra* (available from EMBL/GenBank/DDBJ under accession number X59846) and other mammalian gas6 polypeptides (such as murine gas6, see Manfioletti *et al.*, *supra*). Where the gas6 polypeptide has the amino acid sequence of a gas6 polypeptide found in nature, it is referred to herein as a "native" or "native-sequence" polypeptide regardless of the method by which it is produced (*e.g.*, it can be isolated from an endogenous source of the molecule or produced by synthetic techniques). Gas6 itself can be prepared in a number of ways which have been described in the literature, including U.S. Pat. No. 5,538,861 and WO 96/28548.

Suitable such techniques include isolating gas6 from an endogenous source of this polypeptide (*e.g.*, from serum), peptide synthesis (using a peptide synthesizer) and recombinant techniques (or any combination of these techniques).

"Gas6 antagonist" or "antagonist" refers to a substance that stimulates one or more gas6 receptors (*e.g.*, Rse, Axl, or Mer receptor). The antagonist may be a polypeptide, peptide, or non-peptidyl molecule, such as one with high oral bioavailability, including synthetic organic molecules.

In one embodiment, one of the gas6 receptors is expression cloned and a soluble form of the receptor is made by excising the transmembrane domain from the extracellular domain. The soluble form of the receptor can then be used as an antagonist, or the receptor can be used to screen for small molecules that would antagonize gas6 activity.

A small molecule antagonist is also contemplated herein and constitutes a natural or synthesized non-peptide, organic molecule. Small molecule antagonists are typically identified by screening libraries obtained from soil samples, plant extracts, marine microorganisms, fermentation broth, fungal broth, pharmaceutical chemical libraries, combinatorial libraries (both chemical and biological) and the like.

Gas6 antagonists also encompass peptides, which include amino acid sequences having at least two amino acids, preferably having about 10 to about 25 amino acids, more preferably about 12-25, and most preferably about 15-25 amino acids. The definition includes peptide derivatives, their salts, or optical isomers.

Alternatively, using the gas6 sequence disclosed in U.S. Pat. No. 5,538,861 and WO 96/28548, variants of native gas6 may be synthesized that may act as gas6 antagonists. The receptor binding sites of gas6 can be determined by binding studies and one of them eliminated by standard techniques (deletion or radical substitution), so that the molecule acts as an antagonist. Exemplary variants include fragments of the human gas6 sequence; polypeptides wherein one or more amino acid residues are added at the N- or C-terminus of, or within, the gas6 sequence; one or more amino acid residues are deleted, and optionally substituted by one or more amino acid residues; and derivatives of the above proteins, polypeptides, or fragments thereof, wherein an amino acid residue has been covalently modified so that the resulting product is a non-naturally occurring amino acid, provided that these variants act as gas6 antagonists. Gas6 variants may be prepared, for example, by the methods described in WO 96/28548, such as by site-directed or PCR mutagenesis, or may exist naturally, as in the case of allelic forms and other naturally occurring variants of the translated amino acid sequence set forth in Manfioletti *et al.*, *supra*, that may occur in human and other animal species.

Other examples of gas6 antagonists include neutralizing antibodies to one or more gas6 receptors (such as antibodies to Rse, to Mer, or to Axl), Rse-IgG, Rse extracellular domain (Rse ECD), Axl-IgG, Axl ECD, Mer-IgG, and Mer ECD, as well as any gas6 binding protein displacers such as shed receptors. Preferably, the antagonist is an antibody to a gas6 receptor, and most preferably, the antagonist is a human or humanized antibody to a gas6 receptor.

In order to screen for molecules that act as gas6 antagonists, a candidate molecule can be subjected to one or more of the following functional activity tests/assays:

(a) Receptor activation assays which measure downregulation or activation of receptor tyrosine kinase activity (*e.g.*, western blotting using an anti-phosphotyrosine antibody to determine whether the candidate molecule is able to activate Rse receptor or Mer receptor, see Example 3 of WO 96/28548).

(b) KIRA ELISA to determine Rse or Mer receptor activation-capabilityof the candidate molecule as described in Example 4 of WO 96/28548.

(c) Schwann cell proliferation assay to establish whether or not the candidate molecule is able to enhance Schwann cell proliferation in cell culture. See Example 9 of WO 96/28548.

(d) *In vivo* test of insulin levels and glucose uptake into fat and muscle to determine whether or not the candidate molecule decreases serum insulin levels and increases glucose uptake (see Examples 1 and 2 below).

The term "antibody" is used in the broadest sense and specifically covers single anti-gas6 receptor monoclonal antibodies (antagonist antibodies) and anti-gas6 receptor antibody compositions with polyepitopic specificity.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen.

The monoclonal antibodies herein include hybrid and recombinant antibodies produced by splicing a variable (including hypervariable) domain of an anti-gas6 receptor antibody with a constant domain (*e.g.*, "humanized" antibodies), or a light chain with a heavy chain, or a chain from one species with a chain from another species, or fusions with heterologous proteins, regardless of species of origin or immunoglobulin class or subclass designation, as well as antibody fragments (*e.g.*, Fab, $F(ab')_2$, and Fv), so long as they exhibit the desired biological activity. See, *e.g.*, US Pat No 4,816,567 and Mage & Lamoyi, in Monoclonal Antibody Production Techniques and Applications, pp.79-97 (Marcel Dekker, Inc., New York (1987).

Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, Nature, 256: 495 (1975), or may be made by recombinant DNA methods. U.S. Patent No. 4,816,567. The "monoclonal antibodies" may also be isolated from phage libraries generated using the techniques described in McCafferty *et al.*, Nature, 348: 552-554 (1990), for example.

"Humanized" forms of non-human (*e.g.*, murine) antibodies are specific chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$, or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and

typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody preferably also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

The term "neutralizing antibody" as used herein refers to an antibody that is capable of specifically binding to a gas6 receptor, and which is capable of substantially inhibiting or eliminating the functional activity of a gas6 receptor *in vivo* and/or *in vitro*. Typically a neutralizing antibody will inhibit the functional activity of a gas6 receptor at least about 50%, and preferably greater than 80%, as determined, for example, by KIRA ELISA (see Example 4 of WO 96/28548).

The expression "Rse extracellular domain" or "Rse ECD" when used herein refers to a polypeptide sequence that shares a ligand-binding function of the extracellular domain of the Rse receptor. "Ligand-binding function" refers to the ability of the polypeptide to bind a Rse ligand, such as gas6. Accordingly, it is often not necessary to include the entire extracellular domain since smaller segments are commonly found to be adequate for ligand binding. The term ECD encompasses polypeptide sequences in which the cytoplasmic domain and hydrophobic transmembrane sequence (and, optionally, 1-20 amino acids amino-terminal to the transmembrane domain) of the Rse receptor have been deleted. Generally the ECD of the Rse receptor comprises amino acid residues from about 1-428 of the mature Rse receptor sequence disclosed in Mark *et al.*, *supra*, 1994.

The expression "Mer extracellular domain" or "Mer ECD" when used herein refers to a polypeptide sequence that shares a ligand-binding function of the extracellular domain of the Mer receptor. "Ligand-binding function" refers to the ability of the polypeptide to bind a Mer ligand, such as gas6. Accordingly, it may be unnecessary to include the entire extracellular domain, since smaller segments are commonly found to be adequate for ligand binding. The term ECD encompasses polypeptide sequences in which the cytoplasmic domain and hydrophobic transmembrane sequence (and, optionally, 1-20 amino acids amino-terminal to the transmembrane domain) of the Mer receptor have been deleted. Generally the ECD of the Mer receptor comprises amino acid residues from about 1-499 of the mature human Mer receptor sequence disclosed in Graham *et al.*, Cell Growth Differ., 5: 647 (1994).

The expression "Axl extracellular domain" or "Axl ECD" when used herein refers to a polypeptide sequence that shares a ligand-binding function of the extracellular domain of the Axl receptor. "Ligand-binding function" refers to the ability of the polypeptide to bind an Axl ligand, such as gas6. Accordingly, it is often not necessary to include the entire extracellular domain since smaller segments are commonly found to be adequate for ligand binding. The term ECD encompasses polypeptide sequences in which the cytoplasmic domain and hydrophobic transmembrane sequence (and, optionally, 1-20 amino acids amino-terminal to the transmembrane domain) of the Axl receptor have been deleted. Generally the ECD of the Axl receptor comprises amino acid residues indicated in O'Bryan *et al.*, *supra*, and Janssen *et al.*, *supra*.

Mammalian "Rse receptors" or "Rse receptor protein tyrosine kinases" (*i.e.*, "rPTKs") have been described by Mark *et al.*, *supra*, 1994. When used throughout this application, the expression "Rse receptor" refers to endogenous Rse receptor present in a cell of interest as well as Rse receptor which is present in a cell by virtue of the cell having been transformed with nucleic acid encoding the Rse receptor, for example. Accordingly, the Rse receptor may be an amino acid or covalent variant of one of the native Rse receptors described by Mark *et al.*, *supra*,

1994, provided it is still "functionally active" (*i.e.*, is able to be activated by a Rse ligand such as gas6). The preferred Rse receptor is endogenous human Rse receptor present in the cell membrane of a human cell.

Mammalian "Mer receptors" have been described in Graham *et al.*, Cell Growth Differ., 5: 647 (1994) and Graham *et al.*, Oncogene 10: 2349-2359 (1995). When used throughout this application, the expression "Mer receptor" refers to endogenous Mer receptor present in a cell of interest as well as Mer receptor which is present in a cell by virtue of the cell having been transformed with nucleic acid encoding the Mer receptor, for example. The preferred Mer receptor is endogenous human Mer receptor present in a human cell.

Mammalian "Axl receptors" or "Axl receptor protein tyrosine kinases" (*i.e.*, "Axl rPTKs") have been described by O'Bryan *et al.*, *supra*, and Janssen *et al.*, *supra*. When used throughout this application, the expression "Axl receptor" refers to endogenous Axl receptor present in a cell of interest as well as Axl receptor which is present in a cell by virtue of the cell having been transformed with nucleic acid encoding the Axl receptor, for example. Accordingly, the Axl receptor may be an amino acid or covalent variant of one of the native Axl receptors described by O'Bryan *et al.*, *supra*, and Janssen *et al.*, *supra*, provided it is still "functionally active" (*i.e.*, is able to be activated by an Axl ligand such as gas6). The preferred Axl receptor is endogenous human Axl receptor present in the cell membrane of a human cell.

"Physiologically acceptable" carriers, excipients, or stabilizers are ones which are nontoxic to recipients at the dosages and concentrations employed, and include additives that enhance isotonicity and chemical stability. Often the physiologically acceptable carrier is an aqueous pH-buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, *e.g.*, polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; glycine; amino acids such as glutamic acid, aspartic acid, histidine, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, trehalose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counter-ions such as sodium; non-ionic surfactants such as polysorbates, poloxamers, or polyethylene glycol (PEG); and/or neutral salts, *e.g.*, NaCl, KCl, $MgCl_2$, $CaCl_2$, *etc.*

As used herein, "mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic, and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, sheep, pigs, cows, *etc.* The preferred mammal herein is a human. The term "non-adult" refers to mammals that are from perinatal age (such as low-birth-weightinfants) up to the age of puberty, the latter being those that have not yet reached full growth potential.

As used herein, the term "insulin-resistant disorder" refers to all forms of diabetes and disorders resulting from insulin resistance. These include such conditions as type I and type II diabetes, polycystic ovary disease, hyperinsulinemia, hyperlipidemia, *e.g.*, obese subjects, and severe insulin resistance, such as type A severe insulin resistance, Mendenhall's Syndrome, Werner Syndrome, leprechaunism, lipoatrophic diabetes, and other lipoatrophies. The preferred such disorder is type II diabetes or obesity, most preferably type II diabetes. "Diabetes" itself refers to a progressive disease of carbohydrate metabolism involving inadequate production or utilization of insulin and is characterized by hyperglycemia and glycosuria. Insulin resistance can be determined simply, but crudely, by the ratio of insulin to glucose (high insulin with normal glucose is usually taken as evidence of insulin

resistance). It can be determined more accurately using a euglycemic hyperinsulinemic clamp, which measures the amount of glucose that must be infused to maintain normal glycemia in the presence of increased insulin. The less glucose that is required the more insulin resistant the patient is.

As used herein, the term "treating" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those prone to having the disorder or diagnosed with the disorder or those in which the disorder is to be prevented. Consecutive treatment or administration refers to treatment on at least a daily basis without interruption in treatment by one or more days. Intermittent treatment or administration, or treatment or administration in an intermittent fashion, refers to treatment that is not consecutive, but rather cyclic in nature. The treatment regime herein can be either consecutive or intermittent.

As used herein, the term "hypoglycemic agent" refers to a compound that is useful for regulating glucose metabolism, preferably an oral agent. More preferred herein for human use are insulin, IGF-I, and the sulfonylurea class of oral hypoglycemic agents, which cause the secretion of insulin by the pancreas. Examples include glyburide, glipizide, and gliclazide. In addition, agents that enhance insulin sensitivity or are insulin sensitizing, such as biguanides (including metformin and phenformin) and thiazolidenediones such as REZULIN™ (troglitazone) brand insulin-sensitizing agent, and other compounds that bind to the PPARγ nuclear receptor, are within this definition, and also are preferred. In addition, the definition also encompasses an amylin antagonist such as an antibody directed to amylin.

As used herein, "insulin" refers to any form of insulin from any species, and whether natively or synthetically or recombinantly derived. Preferably it is NPH insulin.

As used herein, "IGF" refers to native insulin-like growth factor-I and native insulin-like growth factor-II as well as natural variants thereof such as brain IGF, otherwise known as des(1-3)IGF-I.

As used herein, "IGF-I" refers to insulin-like growth factor-I from any species, including bovine, ovine, porcine, equine, and human, preferably human, and, if referring to exogenous administration, from any source, whether natural, synthetic, or recombinant. Human native-sequence, mature IGF-I, more preferably without a N-terminal methionine is prepared, *e.g.*, by the process described in EP 230,869 published August 5, 1987; EP 128,733 published December 19, 1984; or EP 288,451 published October 26, 1988. More preferably, this native-sequence IGF-I is recombinantly produced and is available from Genentech, Inc., South San Francisco, CA for clinical investigations.

As used herein, "IGF-II" refers to insulin-like growth factor-II from any species, including bovine, ovine, porcine, equine, and human, preferably human, and, if referring to exogenous administration, from any source, whether natural, synthetic, or recombinant. It may be prepared by the method described in, *e.g.*, EP 128,733.

For purposes herein, a "body sample" is a biological sample extracted or otherwise taken from the mammal suspected of having insulin resistance. It may come from any mammal, but is preferably from a human. Such samples include, but are not limited to, aqueous fluids such as serum, plasma, lymph fluid, synovial fluid, follicular fluid, seminal fluid, amniotic fluid, milk, whole blood, urine, cerebro-spinal fluid, saliva, sputum, tears, perspiration, mucus, tissue culture medium, tissue extracts, and cellular extracts. Preferred such samples are serum and plasma.

2. Modes for Carrying Out the Invention

The present invention, in one aspect, provides a method for treating insulin-resistant disorders using a gas6 antagonist. For gas6 antagonists, any antagonist as defined above may be used. However, antibodies are preferred, most preferably human or humanized antibodies. Polyclonal antibodies directed toward gas6 receptors generally are raised in animals by multiple subcutaneous or intraperitoneal injections of gas6 and an adjuvant. It may be useful to conjugate a gas6 receptor or a peptide fragment thereof to a carrier protein that is immunogenic in the species to be immunized, such as keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor, using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (conjugation through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N = C = NR$, where R and R^1 are different alkyl groups.

Animals are immunized with such conjugates of gas6 receptor and carrier protein by combining 1 mg or 1 μg of conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5th to 1/10th the original amount of conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later animals are bled and the serum is assayed for anti-gas6-receptor antibody titer. Animals are boosted until the antibody titer plateaus. Preferably, the animal is boosted by injection with a conjugate of the same gas6 receptor with a different carrier protein and/or through a different cross-linking agent. Conjugates of gas6 receptor and a suitable carrier protein also can be made in recombinant cell culture as fusion proteins. Also, aggregating agents such as alum are used to enhance the immune response.

Monoclonal antibodies directed toward gas6 receptor are produced using any method which provides for the production of antibody molecules by continuous cell lines in culture. Examples of suitable methods for preparing monoclonal antibodies include the original hybridoma method of Kohler and Milstein, *supra*, and the human B-cell hybridoma method by Kozbor, Immunol., 133: 3001 (1984) and Brodeur *et al.*, Monoclonal Antibody Production Techniques and Applications, pp.51-63 (Marcel Dekker, Inc., New York, 1987).

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be performed following methods known in the art (Jones *et al.*, Nature, 321: 522-525 (1986); Riechmann *et al.*, Nature, 332: 323-327 (1988); and Verhoeyen *et al.*, Science, 239: 1534-1536 (1988)), by substituting rodent complementarity-determining regions (CDRs) for the corresponding regions of a human antibody.

Alternatively, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, for example, Jakobovits *et al.*, PNAS USA, 90: 2551-2555 (1993); Jakobovits *et al.*, Nature, 362: 255-258 (1993); and Bruggermann *et al.*, Year in Immuno., 7: 33 (1993). Human antibodies can also be produced in phage-display libraries. Hoogenboom *et al.*, J. Mol. Biol., 227: 381 (1991); and Marks *et al.*, J. Mol. Biol., 222: 581 (1991).

Normally, the cells will be treated with the gas6 antagonist. However, the invention contemplates using gene therapy for treating a mammal, using nucleic acid encoding the gas6 antagonist, if it is a protein. Generally, gene therapy is used to decrease the levels of endogenous gas6 in the mammal. Nucleic acids that encode the gas6 antagonist such as antibodies can be used for this purpose.

There are two major approaches to getting the nucleic acid (optionally contained in a vector) into the patient's cells for purposes of gene therapy: *in vivo* and *ex vivo*. For *in vivo* delivery, the nucleic acid is injected directly into the patient, usually at the site where the gas6 antagonist is required. For *ex vivo* treatment, the patient's cells are removed, the nucleic acid is introduced into these isolated cells, and the modified cells are administered to the patient either directly or, for example, encapsulated within porous membranes that are implanted into the patient. See, *e.g.*, U.S. Pat. Nos. 4,892,538 and 5,283,187. There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro* or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, *etc.* A commonly used vector for *ex vivo* delivery of the gene is a retrovirus.

The currently preferred *in vivo* nucleic acid transfer techniques include transfection with viral vectors (such as adenovirus, Herpes simplex I virus, or adeno-associated virus) and lipid-based systems (useful lipids for lipid-mediated transfer of the gene are DOTMA, DOPE and DC-Chol, for example; see, *e.g.*, Tonkinson *et al.* Cancer Investigation, 14(1): 54-65 (1996)). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell-surface membrane protein or the target cell, a ligand for a receptor on the target cell, *etc.* Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.*, capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, J. Biol. Chem., 262: 4429-4432 (1987); and Wagner *et al.*, Proc. Natl. Acad. Sci. USA, 87: 3410-3414 (1990). For review of the currently known gene marking and gene therapy protocols, see Anderson *et al.*, Science, 256: 808-813 (1992). See also WO 93/25673 and the references cited therein, and U.S. Pat. No. 5,681,746.

Characterization and construction of chimeras and immunoadhesins of gas6 or of receptors therefor, and epitope-tagged gas6, are described in detail in WO 96/28548.

For exogenous administration, the gas6 antagonist is directly administered to the mammal by any suitable technique, including infusion and injection. The specific route of administration will depend, *e.g.*, on the medical history of the patient, including any perceived or anticipated side effects using gas6 antagonist, and the particular disorder to be corrected. Examples of parenteral administration include subcutaneous, intramuscular, intravenous, intraarterial, and intraperitoneal administration. Most preferably, the administration is by continuous infusion (using, *e.g.*, slow-release devices or minipumps such as osmotic pumps or skin patches), or by injection (using, *e.g.*, intravenous or subcutaneous means). Preferably, the administration is by subcutaneous injection. The administration may also be as a single bolus or by slow-release depot formulation. Delivery of gas6 antagonist by injection will be the preferred form of administration for treating insulin-resistant disorders.

The gas6 antagonist composition to be used in the therapy will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with gas6 antagonist), the particular disorder, the site of delivery of the gas6 antagonist composition, the method of administration, the scheduling of administration, the presence of other hypoglycemic agents, and other factors known to practitioners. The "effective amount" of gas6 antagonist for purposes herein is thus determined by such considerations and must be an amount that results in bioavailability of the drug to the mammal and an effect of increasing insulin levels in the serum.

As a general proposition, the total pharmaceutically effective amount of the gas6 antagonist administered parenterally per dose will be in the range of from about 10 µg/kg/day to 200 µg/kg/day of gas6 antagonist based on kg of patient body weight, although, as noted above, this will be subject to a great deal of therapeutic discretion. Where possible, it is desirable to determine appropriate dosage ranges first *in vitro*, for example, using assays for measuring insulin and glucose levels which are known in the art, and then in suitable animal models, from which dosage ranges for human patients may be extrapolated. In one embodiment of the invention, a pharmaceutical composition effective in treating diabetes will provide a local gas6 antagonist concentration *in vivo* of between about 0.1 and 10 ng/ml. In another specific preferred embodiment for treatment of diabetes in humans, the dose of gas6 antagonist is from about 1 to 10 mg twice per day, more preferably from about 20 to 80 µg/kg/injection (*i.e.*, from about 1.5 to 6 mg) twice a day subcutaneously.

Although injection is preferred, an infusion device may also be employed for continuous SC infusions. An intravenous bag solution may also be employed. The key factor in selecting an appropriate dose is the result obtained, as measured by increases in endogenous insulin levels, or by other criteria for measuring treatment of insulin-resistant disorders as defined herein as are deemed appropriate by the practitioner.

If a small molecule antagonist is used as a gas6 antagonist, it may have cyclical effects and require, for efficacy, an administration regimen appropriate thereto. For a peptide, one preferred administration is a chronic administration of about two times per day for 4-8 weeks to reproduce the effects of a natural antagonist to gas6. A small peptide may be administered orally.

The gas6 antagonist is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, *e.g.*, films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman *et al.*, Biopolymers, 22, 547-556 (1983)), poly(2-hydroxyethyl methacrylate) (Langer *et al.*, J. Biomed. Mater. Res., 15: 167-277 (1981), and Langer, Chem. Tech., 12: 98-105 (1982)), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release gas6 antagonist compositions also include liposomally entrapped gas6 antagonist. Liposomes containing gas6 antagonist are prepared by methods known *per se*: DE 3,218,121; Epstein *et al.*, Proc. Natl. Acad. Sci. U.S.A., 82: 3688-3692 (1985); Hwang *et al.*, Proc. Natl. Acad. Sci. U.S.A., 77: 4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appln. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; WO 91/04014; and EP 102,324. Ordinarily, the liposomes are of the small (from about 200 to 800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the maximal gas6 antagonist therapy.

In another embodiment, the gas6 antagonist used for therapeutic effect is gas6 antagonist covalently joined to another protein, such as an immunoglobulin domain (for example, to produce a chimera of anti-gas6 antibody and IgG). Gas6 antagonist also may be covalently linked to nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in WO 95/32003 or U.S. Pat. Nos. 4,179,337; 4,301,144; 4,496,689; 4,640,835; 4,670,417; or 4,791,192.

For parenteral administration, in one embodiment, the gas6 antagonist is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a physiologically acceptable carrier as defined above, *i.e.*, one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

The gas6 antagonist typically is formulated in such vehicles at a pH of from about 4.5 to 8. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of salts of the gas6 antagonist. The final preparation may be a stable liquid or lyophilized solid.

Gas6 antagonist to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (*e.g.*, 0.2 micron membranes). Therapeutic gas6 antagonist compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The gas6 antagonist ordinarily will be stored in unit or multi-dose containers, for example, sealed ampules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-mL vials are filled with 5 mL of sterile-filtered 1% (w/v) aqueous gas6 antagonist solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized gas6 antagonist using bacteriostatic Water-for-Injection. The final liquid formulation, whether always a liquid or reconstituted, is preferably stored at a temperature of from about 2 to 8°C for up to about four weeks or longer.

Gas6 antagonist optionally is combined with or administered in concert with an effective amount of one or more other hypoglycemic agents to achieve a desired therapeutic effect. Preferably, the treatment composition contains a prophylactically or therapeutically effective amount of the gas6 antagonist in combination with a prophylactically or therapeutically effective amount of a hypoglycemic agent that acts synergistically or additively to enhance or complement the prophylactic or therapeutic effect of the gas6 antagonist. For example, gas6 antagonist may be used together with insulin or an insulin-like growth factor (*e.g.*, IGF-I or IGF-II) or a thiazolidenedione, or a sulfonylurea, or another hypoglycemic agent to achieve an additive or synergistic glucose-lowering effect in muscle or fat cells, wherein the term "synergistic" means that the effect of the combination of gas6 antagonist with a second substance is greater than that achieved with either substance used individually. The hypoglycemic agent can be administered sequentially or simultaneously with the gas6 antagonist. In addition, other means of manipulating serum glucose levels, such as regimens of diet or exercise, are also considered to be combination treatments as part of this invention.

The hypoglycemic agent is administered to the mammal by any suitable technique, including parenterally, intranasally, orally, or by any other effective route. Most preferably, the administration is by the oral route if the hypoglycemic agent is not a cytokine or other polypeptide. For example, MICRONASE™ tablets (glyburide) marketed by Upjohn in 1.25-, 2.5-, and 5-mg tablet concentrations are suitable for oral administration. The usual

maintenance dose for type II diabetics, placed on this therapy, is generally in the range of from about 1.25 to 20 mg per day, which may be given as a single dose or divided throughout the day as deemed appropriate. Physician's Desk Reference, 2563-2565 (1995). Other examples of glyburide-based tablets available for prescription include GLYNASE™-branddrug (Upjohn) and DIABETA™- brand drug (Hoechst-Roussel). GLUCOTROL™ (Pratt) is the trademark for a glipizide (1-cyclohexyl-3-(p-(2-(5-methylpyrazine carboxamide)ethyl)phenyl)sulfonyl)urea) tablet available in both 5- and 10-mg strengths and is also prescribed to type II diabetics who require hypoglycemic therapy following dietary control or in patients who have ceased to respond to other sulfonylureas. Physician's Desk Reference, 1902-1903 (1995).

Other hypoglycemic agents than sulfonylureas, such as the biguanides (*e.g.*, metformin and phenformin) or thiazolidinediones (*e.g.*, troglitozone), or other drugs affecting insulin action may also be employed. If a thiazolidinedione is employed with the gas6 antagonist, it is used at the same level as currently used or at somewhat lower levels, which can be adjusted for effects seen with the gas6 antagonist alone or together with the dione. The typical dose of troglitazone (REZULIN™) employed by itself is about 100-1000 mg per day, more preferably 200-800 mg/day, and this range is applicable herein. See, for example, Ghazzi *et al.*, Diabetes, 46: 433-439 (1997). Other thiazolidinedionesthat are stronger insulin-sensitizing agents than troglitazone would be employed in lower doses.

In addition, an amylin antagonist may be administered in conjunction with the gas6 antagonist, at least for treating type 2 diabetes mellitus, as described in U.S. Pat. No. 5,716,619.

If insulin is also administered, it can be any formulation of insulin, but is preferably NPH insulin. The ratio of insulin to gas6 antagonist in this formulation by weight is generally from about 10:1 to 1:50, preferably from about 1:1 to 1:20, more preferably from about 1:1 to 1:10, still more preferably, from about 1:1 to 1:5, and most preferably from about 1:1 to 1:3. The typical dose of insulin is from about 0.5 to 500 units/day of NPH insulin. For treatment of diabetes in humans, the dose of NPH insulin is from about 5 to 50 units/injection (*i.e.*, from about 0.2 to 2 mg) twice a day subcutaneously. Further information on dosing NPH insulin can be found in Diabetes Mellitus - Theory and Practice, fourth edition, Harold Rifkin, MD, Ed. (Elsevier, New York, 1990), Chapters 29 and 30.

In an embodiment for administering the combination of gas6 antagonist and IGF-I and/or insulin, the insulin and gas6 antagonist administration is continuous and the IGF-I is administered to the mammal in an intermittent fashion so as to sustain its biological response in the treatment of an insulin-resistant disorder. This is accomplished usually by administering a therapeutically effective amount of the gas6 antagonist, IGF-I, and/or insulin to the mammal to provide an exposure to gas6 antagonist, IGF-I, and/or insulin for a period of time that provides the maximum biological response in the mammal, then discontinuing the administration of the IGF-I (but not the insulin or gas6 antagonist) for a period of time equal to or less than the time period during which the IGF-I was previously administered, then administering a therapeutically effective amount of IGF-I (with insulin and gas6 antagonist administration continuing) to the mammal to provide an exposure to gas6 antagonist, IGF-I, and/or insulin for a period of time that provides the maximum biological response in the mammal, then discontinuing the administration of the IGF-I (but not the insulin or gas6 antagonist) for a period of time equal to or less than the time period during which the IGF-I was just previously administered, and repeating this pattern of administration and discontinuance of administration of IGF-I for as long as necessary to achieve or maintain sustained biological response in the mammal.

In a preferred formulation, if IGF-I is employed, the amount of IGF-I is from about 8 to 12 mg/mL, the amount of sodium chloride is from about 5 to 6 mg/mL, the stabilizers are benzyl alcohol in an amount of from about 8 to 10 mg/mL and/or phenol in an amount of from about 2 to 3 mg/mL, and the buffer is about 50 mM sodium acetate so that the pH is about 5.4. More specifics on types of formulations with NPH insulin and IGF-I and how they can be prepared can be found in International Application, publication WO98/06423, published 19 FEB 1998, the disclosures of which are incorporated herein by reference. These specifics can be used to devise combinations suitable for treatment of insulin-resistant disorders constituting gas6 antagonist with IGF-I alone, with insulin alone, or with the combination of IGF-I and insulin, preferably NPH insulin, but not limited thereto.

Also, the formulation herein containing gas6 antagonist and an IGF is suitably administered along with an IGF binding protein, for example, one of those currently known, *i.e.*, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5, or IGFBP-6, or with the ALS of the IGF binding complex. Such proteins may be administered separately or as a complex with the IGF, preferably IGF-I. The IGF may also be coupled to a receptor, antibody, or antibody fragment for administration. The preferred binding protein for IGF-I herein is IGFBP-3, which is described by U.S. Pat. No. 5,258,287 and Martin and Baxter, J. Biol. Chem., 261: 8754-8760 (1986). This glycosylated IGFBP-3 protein is an acid-stable component of about 53 Kd on a non-reducing SDS-PAGE gel of a 125-150 Kd glycoprotein complex found in human plasma that carries most of the endogenous IGFs and is also regulated by GH.

The administration of the IGF binding protein with IGF-I and gas6 antagonist may be accomplished by the method described in U.S. Pat. No. 5,187,151. Briefly, the IGF-I and IGFBP are administered in effective amounts by subcutaneous bolus injection in a molar ratio of from about 0.5:1 to 3:1, preferably about 1:1; and the gas6 antagonist is either already present with the IGF-I or administered separately.

Kits and articles of manufacture containing materials useful for treating an insulin-resistant disorder are also contemplated for this invention. The kit or article of manufacture comprises a container with a label. Suitable containers include, for example, bottles, vials, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating an insulin-resistant disorder such as diabetes. The active agent in the composition is gas6 antagonist. The label on the container indicates that the composition is used for treating an insulin-resistant disorder, and may also indicate directions for either *in vivo* or *in vitro* use, such as those described above. The composition may optionally also contain a hypoglycemic agent, such as insulin, or an IGF, a sulfonylurea, or a thiazolidinedione.

The kit of the invention may comprise the container described above and a second container comprising a buffer. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

For example, a typical kit would comprise a container, preferably a vial, for the gas6 antagonist formulation comprising gas6 antagonist in a pharmaceutically acceptable buffer, and instructions, such as a product insert or label, directing the user how to administer the pharmaceutical formulation. Preferably, the pharmaceutical formulation is for treating diabetes.

Also part of this invention is an article of manufacture, comprising a first container as described above having a label thereon and containing a first composition comprising a gas6 antagonist and a second container having a label thereon and containing a second composition comprising a hypoglycemic agent;

wherein the compositions are effective for treating an insulin-resistant disorder and the labels on said containers indicate that the compositions can be used for treating an insulin-resistant disorder.

For example, a typical kit would comprise a container, preferably a vial, for the gas6 antagonist formulation comprising gas6 antagonist in a pharmaceutically acceptable buffer, a container, preferably a vial, comprising pharmaceutically acceptable insulin, such as NPH insulin, or IGF-I and instructions, such as a product insert or label, directing the user to combine the contents of the two containers, *i.e.*, the two formulations, to provide a pharmaceutical formulation. Preferably, the pharmaceutical formulation is for treating diabetes. Also, preferably, the user will be instructed to combine the contents of the containers, *i.e.*, the two formulations, in a syringe for immediate injection.

If the second container contains IGF-I, the IGF-I composition preferably additionally comprises sodium chloride and benzyl alcohol or phenol, or both, in the buffer at a pH of from about 5.0 to 5.5. In another preferred embodiment, the container with IGF-I comprises from about 8 to 12 mg/mL of IGF-I, from about 5 to 6 mg/mL of sodium chloride, from about 8 to 10 mg/mL of benzyl alcohol or from about 2 to 3 mg/mL of phenol, or both from about 8 to 10 mg/mL of benzyl alcohol and from about 2 to 3 mg/mL of phenol, in an about 50 mM sodium acetate buffered solution at a pH of about 5.4. More preferably, this container further comprises from about 1 to 3 mg/mL polysorbate.

In another aspect, the invention provides a method for determining or diagnosing if a mammal has an insulin-resistant disorder. This method involves assaying the level of endogenous gas6 in a body sample derived from the mammal and ascertaining if that level is elevated over the level in a comparable mammal that does not have an insulin-resistant disorder. A comparable mammal is a mammal of the same species as the mammal being diagnosed and preferably of an age that reflects the same stage of life as that of the mammal being diagnosed. For example, a young human adult would be compared to another young human adult.

In one embodiment, the level of endogenous gas6 in the mammal is measured using an antibody to gas6 under conditions that promote binding of the antibody to the gas6 in the sample. The antibodies may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, Monoclonal Antibodies: A Manual of Techniques, pp. 147-158 (CRC Press, Inc., 1987), preferably an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

Competitive binding assays rely on the ability of a labeled standard (which may be a native-sequence gas6 polypeptide or an immunologically reactive portion thereof) to compete with the test sample analyte (gas6) for binding with a limited amount of antibody. The amount of gas6 in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies generally are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the endogenous gas6 to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. David and Greene, U.S. Patent No. 4,376,110. The second antibody may

itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme (*e.g.*, horseradish peroxidase).

Another immunoassay, RIA, has been developed and is well known in the art and useful for detecting gas6 levels. See, for example, Bala and Bhaumick, J. Clin. Endocrin. and Metabol., 49: 770-777 (1979) and Zapf *et al.*, J. Clin. Invest., 68: 1321-1330 (1981).

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All literature and patent citations herein are incorporated by reference.

EXAMPLE 1

I. Materials and Methods

A. Production of murine gas6

The sequence of murine gas6 is provided in Fig.2 of U.S. Pat. No. 5,538,861 and in the Dayhoff database, and the sequence of the 2573-bp gene encoding murine gas6 is provided in the Genbank database as MMGAS6 (M.musculus GAS 6 mRNA associated with growth-arrest). The gene is also described by Schneider *et al.*, *supra*, and Manfioletti *et al.*, *supra*.

A mouse brain cDNA lambda library (Clontech ML1042) was screened with ^{32}P- labeled oligonucleotide probes to the 5' and 3' ends of murine Gas6 cDNA. Clones that were positive for both probes were isolated and characterized. Conditions used in this cloning, including PCR techniques, are described in Mark *et al.*, *supra*. One of these clones was sequenced and determined to be full length.

The murine Gas6 cDNA so obtained was subcloned into a mammalian expression vector and this plasmid was transfected into CHO-dp12 cells. Clones positive for DHFR selection were isolated and characterized by their ability to activate the human Rse receptor in a phosphorylation assay. For expression of murine Gas6, cells were conditioned in serum-free medium containing vitamin K at a concentration of 1-5 μg/ml. The gas6 can be purified by passing the medium containing gas6 through a column to which is adhered a fusion of Rse receptor-IgG.

B. *In vivo* Experiments

Twelve-week old C57Bl6 female mice (Charles Rivers Labs, Raleigh) were group housed under conditions of standard temperature and lighting and fed normal rodent chow and tap water *ad libitum*. The mice were weighed on the day of the study and randomized into four groups of five. The mice were fasted 4-7 hours and injected i.v. via lateral tail vein with 0.3 ml saline, murine gas6 (40 mg/kg), insulin (Iletin 2^{TM}, Eli Lilly) (2U/kg), or murine gas6 and insulin at the aforementioned doses.

Immediately following the i.v. dose, the mice were administered an i.p. bolus of 0.1 ml saline containing 0.2 micromoles of 5 μCi ^{3}H-2-deoxyglucose (New England Nuclear, Boston, MA) and 2 μCi ^{14}C(U)-sucrose (Amersham, Arlington Heights, IL). At 30 minutes mice were exanguinated by cardiac puncture under CO_2 anesthesia. Blood glucose was measured using freshly collected blood with a ONE-TOUCH^{TM} blood glucose monitor (Lifescan). Serum was analyzed for ^{3}H-2-deoxyglucose and ^{14}C-sucrose counts. Serum insulin levels were measured by radioimmunoassay (LINCO, St. Charles, MO).

Tissues, including uterine fat, subcutaneous fat, retroperitoneal fat, brown adipose tissue, soleus muscle, quadricep muscle, diaphram, heart, lung, liver, kidney, spleen, and brain, were removed. Weighed aliquots (5-100

mg) were solubilized with 1 ml SOLVABLE™ (Packard) and incubated at 55°C until clear (6-8 hours). A total of 10 ml of scintillation solution (HIONICFLOUR™, Packard) was added, and double isotope counting was performed in a BECKMAN™ liquid scintillation counter. Corrections for tissue ^{3}H-2-deoxyglucose in extracellular fluid were made by dividing tissue ^{3}H-2-deoxyglucose concentration by ^{14}C-sucrose concentration.

II. Results

Insulin performed as expected, decreasing serum ^{3}H-2-deoxyglucose (70 vs. 222 DPM/µl;insulin vs. saline-treated;$p<0.0001$) and total blood glucose (124 vs. 40 mg/dL; insulin vs. saline-treated; $p<0.0001$). Insulin increased intracellular ^{3}H-2-deoxyglucose in all fat and muscle.

Blood glucose levels were increased 19% in murine gas6-treated mice (124 vs. 148mg/dL; $p<0.003$) as compared to saline-treated controls. The blood glucose levels were not significantly altered in mice treated with murine gas6 plus insulin as compared to those treated with insulin alone (37.5 vs 40.0 mg/dL, respectively; $p<0.7216$). However, serum insulin levels in mice treated with murine gas6 plus insulin were 7-fold higher at thirty minutes than those treated with insulin alone (18.0 vs 4.3 ng/ml; $p<0.001$).

The lack of an increased hypoglycemic response in the face of prolonged insulin lifetime suggests that murine gas6 treatment rendered the mice resistant to insulin. Accordingly, fat and muscle intracellular ^{3}H-2-deoxyglucose levels in mice treated with murine gas6 plus insulin as compared to those treated with insulin alone were also unaltered by the murine gas6-induced reduction in insulin clearance. Retroperitoneal fat pads from mice treated with murine gas6 in combination with insulin accumulated 326 DPM intracellular ^{3}H-2-deoxyglucose per mg tissue as compared to 350 DPM per mg in mice treated with insulin alone ($p<0.95$). In the soleus muscle, the values for intracellular ^{3}H-2-deoxyglucosewere 300 vs 276 DPM/mg; murine gas6 plus insulin vs. insulin alone; $p<0.1483$.

EXAMPLE 2

I. Methods

To investigate the murine gas6 dose range and duration for reduction in insulin clearance, twelve-week old C57Bl6 female mice (Charles Rivers Labs, Raleigh) were group housed under conditions of standard temperature and lighting and fed normal rodent chow and tap water *ad libitum*. The mice were weighed on the day of the study and randomized into six groups of five. Mice were fasted 4-7 hours and injected i.v. via lateral tail vein with 0.1 ml insulin (2U/kg) alone (Iletin 2™, Eli Lilly) or in combination with murine gas6 prepared as described in Example 1 (13, 4.5, 1.5, or 0.5 mg/kg). Blood was sampled via cardiac stick at 30 minutes for mice dosed with 13 and 4.5 mg/kg of murine gas6. Blood was sampled via retroorbital sinus at 10, 30, 60, 150, and 300 minutes for mice dosed with 1.5 and 0.5mg/kg of murine gas6. Appropriate controls were used for blood sampling of mice treated with insulin alone. Blood glucose was measured via a ONE-TOUCH™ blood glucose moniter (Lifescan). Serum insulin levels were measured by radioimmunoassay, (LINCO, St. Charles, MO).

II. Results

Even at the lowest murine gas6 dose tested (0.5 mg/kg), serum insulin levels remained two-fold higher at 30 minutes as compared to those treated with insulin alone (5.0 vs 2.5 ng/ml; $p<0.009$). In mice dosed with 1.5 mg/kg of murine gas6 the insulin levels remained high out to 60 minutes. By 300 minutes there was no difference in serum insulin concentration but mice dosed with 1.5 mg/kg murine gas6 had 33% higher blood glucose levels than those treated with insulin alone (105 vs 79; $p<0.007$).

III. Conclusion

It was found that mice treated with various doses of gas6 in combination with insulin have a higher insulin level than mice treated with only insulin, yet the glucose levels are the same in both cases and the uptake of glucose into fat and muscle is no different. From these data, it would be expected that an antagonist to gas6, such as a human or humanized antibody to human gas6, would act in a range of doses to decrease the insulin resistance in mammals, such as humans, that are in an insulin-resistant state, and therefore would act as a hypoglycemic agent.

WHAT IS CLAIMED IS:

1. A method for treatment of an insulin-resistant disorder comprising administering to a mammal in need of such treatment an effective amount of a composition comprising a gas6 antagonist.

2. The method of claim 1 wherein the insulin-resistant disorder is obesity or diabetes.

3. The method of claim 1 wherein the insulin-resistant disorder is type II diabetes.

4. The method of claim 1 wherein the mammal is a human.

5. The method of claim 1 wherein the gas6 antagonist is to human gas6 polypeptide.

6. The method of claim 5 wherein the gas6 polypeptide is a native-sequence gas6 polypeptide.

7. The method of claim 1 wherein the gas6 antagonist is an antibody to a gas6 receptor.

8. The method of claim 7 wherein the gas6 antagonist is a human or humanized antibody to a gas6 receptor.

9. The method of claim 1 additionally comprising administering an effective amount of a hypoglycemic agent to the mammal.

10. The method of claim 9 wherein the hypoglycemic agent is present in the composition containing the gas6 antagonist.

11. The method of claim 9 wherein the hypoglycemic agent is administered separately from the gas6 antagonist.

12. The method of claim 9 wherein the hypoglycemic agent is insulin, an insulin-like growth factor, a thiazolidinedione, or a sulfonylurea.

13. The method of claim 12 wherein the hypoglycemic agent is insulin or insulin-like growth factor-I.

14. A composition comprising a gas6 antagonist and a hypoglycemic agent.

15. The composition of claim 14 further comprising a carrier.

16. The composition of claim 14 wherein the hypoglycemic agent is a thiazolidinedione or sulfonylurea.

17. An article of manufacture, comprising:

a container;

a label on said container; and

a composition contained within said container comprising a gas6 antagonist;

wherein the composition is effective for treating an insulin-resistant disorder and the label on said container indicates that the composition can be used for treating an insulin-resistant disorder.

18. The article of claim 17 wherein the gas6 antagonist is an antibody against a gas6 receptor.

19. The article of claim 18 wherein the gas6 antagonist is a human or humanized antibody against a gas6 receptor.

20. The article of claim 17 wherein the disorder is diabetes.

21. The article of claim 20 wherein the composition further comprises a hypoglycemic agent.

22. An article of manufacture, comprising:

a first container;

a label on said first container;

a first composition contained within said first container comprising a gas6 antagonist;

a second container;

a label on said second container;

a second composition contained within said second container comprising a hypoglycemic agent;

wherein the compositions are effective for treating an insulin-resistant disorder and the labels on said containers indicate that the compositions can be used for treating an insulin-resistant disorder.

23. The article of claim 22 wherein the disorder is diabetes and the hypoglycemic agent is insulin, an insulin-like growth factor, a thiazolidinedione, or a sulfonylurea.

24. A method for determining if a mammal has an insulin-resistant disorder comprising measuring the level of endogenous gas6 in a body sample of the mammal and ascertaining if the level is elevated over the level in a comparable mammal that does not have an insulin-resistant disorder.

25. The method of claim 24 wherein measuring the level of endogenous gas6 is accomplished using an antibody to gas6.

26. The method of claim 25 wherein the measuring is conducted using an ELISA or RIA.

INTERNATIONAL SEARCH REPORT

Intern. ..ial Application No
PCT/US 99/07093

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/395 A61K31/00 A61K38/00 //C07K16/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AUGUSTINE, K. A. (1): "Ectopic expression of human Axl in the myeloid-monocytic lineage causes noninsulin-dependent diabetes mellitus in transgenic mice." FASEB JOURNAL, (MARCH 20, 1998) VOL. 12, NO. 5, PP. A1103. MEETING INFO.: ANNUAL MEETING OF THE PROFESSIONAL RESEARCH SCIENTISTS ON EXPERIMENTAL BIOLOGY 98, PART II SAN FRANCISCO, CALIFORNIA, USA APRIL 18-22, 1998 FEDERATION OF AMERICAN SOCIETIES FOR, XP002109269 the whole document	1-3,5,6, 17

A	WO 97 26005 A (GENENTECH INC) 24 July 1997 (1997-07-24) page 1, line 6-11 page 4, line 27-30 page 14, line 23 - page 18, line 16	1-26

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 July 1999	29/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Covone, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internat. ial Application No
PCT/US 99/07093

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication.where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 28548 A (GENENTECH INC ;CHEN JIAN (US); HAMMONDS R GLENN (US); GODOWSKI PAU) 19 September 1996 (1996-09-19) cited in the application page 1, line 4-7 page 33, line 11-18 page 34, line 20 - page 36, line 24 page 52, line 19 - page 53, line 21	1-26

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/07093

Box I Observations wher certain claims were found unsearchable (Continuation of Item 1 f first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-13 and 24-26
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99 /07093

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-13 are directed to a method of treatment of the human/animal body and claims 24-26 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/ composition.

Continuation of Box I.1

Claims Nos.: 1-13 and 24-26

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy
Rule 39.1(iv) PCT - Diagnostic method practised on the human or animal body

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna al Application No
PCT/US 99/07093

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9726005 A	24-07-1997	AU 1570997 A	11-08-1997
WO 9628548 A	19-09-1996	AU 5183696 A	02-10-1996
		CA 2214629 A	19-09-1996
		EP 0815224 A	07-01-1998
		JP 10505507 T	02-06-1998